THE SOLUBILIZATION OF MITOCHONDRIAL PROTEIN BY NONIONIC SURFACE ACTIVE AGENTS

by

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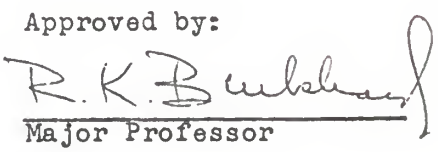

Major Professor

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INTRODUCTION

The number of methods devised for extraction of enzyme systems from animal tissue has greatly increased during the past few decades. These methods have been reviewed by Morton and include ultrasonic vibration, repeated freezing and thawing, extraction by means of dilute aqueous solutions of salts or buffers, drying with acetone, treatment with alcohols, extraction with aqueous solutions of detergents and related compounds, and treatment with hydrolytic enzymes (23).

Until recently mitochondrial enzymes were regarded as insoluble and inseparable from the mitochondrial particle. It is now known that many mitochondrial enzymes pass freely into solution once the mitochondrial membrane has been ruptured (8). However, Criddle and co-workers have found that some enzymes are firmly bound to a structural framework of the mitochondria (6). Dixon and Webb (8) state that this binding probably involves a lipoprotein complex; therefore, special methods are required for the extraction of enzymes of this nature. Green (12) has found that relatively few useful reagents effectively fragment the intact mitochondria, releasing the enzymes of the electron transport chain. According to him, the detergents (dodecyl sulfate, cetyldimethylethyl ammonium bromide), bile acids (cholate, deoxycholate in the presence of salt), lower chain alcohols (butyl, isobutyl, t-amyl), and hydrocarbons (cyclohexane, isooctane, petroleum ether) are the only known classes of compounds which can be used effectively for fragmentation without sacrifice of

enzymatic activity.

Knowledge of some of the properties of surfactant solutions is relevant in order to better comprehend the forthcoming investigations. Adamson (1) states that surfactant solutions generally are found to exhibit an interesting and rather special set of properties characteristic of what are called colloidal electrolytes. For solutions of a typical colloidal electrolyte such as sodium dodecyl sulfate, it has been shown that striking alterations in the various physical properties occur in the region of what is called the critical micelle concentration, or c.m.c. The near constancy of the osmotic pressure and the sudden increase in the light scattering indicate that the system is becoming colloidal in nature. With the aid of other physical measurements, it has been shown that in the region of the c.m.c., the long-chain electrolyte first begins to aggregate into fairly large, charged units. These units are commonly called micelles, and the concentration at which the micelles first begin to form is defined as the c.m.c. The actual structure of the micelle has been disputed in the literature for several decades, but the two most commonly accepted possibilities are the spherical model proposed by Hartley (15), and the lamellar micelle of McBain (22). The types and mechanisms of solubilization by substances capable of forming micelles were extensively reviewed by Klevens (18). He found that when organic compounds such as dyes, benzene, alkanes, etc., were being solubilized, there were three possibilities for the explanation of this type of solubilization:

the possibility of adsorption on the surface of the micelle (dimethyl phthalate in soap solutions); actual incorporation into the hydrocarbon center of the micelle (ethyl benzene in potassium laurate solutions); and incorporation by penetrating into the lamellar region of the micelle (1-heptanol in potassium myristate solutions).

Nothing definite has been established about the actual mechanism involved in the solubilization of protein by surfactants, but it appears to be quite different from that described earlier for simple organic compounds. Criddle and co-workers (6) published a paper in which they interpreted some of the physical properties and structural characteristics of the component proteins of the mitochondria. In this paper they proposed a mechanism to explain why ionic surfactants, such as sodium dodecyl sulfate, are so effective in fragmenting (and in a sense solubilizing) components of the electron transport chain of beef heart mitochondria. While it is still evident that lipid plays a major functional role in stabilizing the structure of the mitochondrion, their studies demonstrate that there are strong protein-protein interactions, hydrophobic in nature, which may be the most important determinant of mitochondrial structure, suggesting that lipid-protein binding is a quantitatively less important factor. Kauzmann (16) has shown that the hydrophobic bonding between non-polar groups of proteins would lead to increased stability of the system. This type of binding was thought to be stabilized mainly by the increased entropy of the

water molecules which were previously in the vicinity of the non-polar groups, which are now coalesced. Criddle et al. (6) presented evidence from their own work to support the conclusion that these interactions are largely hydrophobic in nature. They found that anionic detergents functioned as the most effective agents for breaking up hexameric cytochrome c_1 or in solubilizing structural protein. They stated that these reagents have the capability of entering the non-polar regions that bind the complex together and competing for the individual hydrophobic binding sites. Also, anionic detergents would introduce a large number of like charges, with a resultant charge repulsion. It might be noted that they linked the inability of nonionic detergents, such as Triton X-100, to effectively break up the various complexes to their lack of ability of introducing charged sites. To summarize, these two factors, charge repulsion and competition for hydrophobic binding sites, were thought by Criddle et al. to bring about the breakdown of the various complexes into their component parts.

It appears from their findings that the formation of a micelle plays little or no part in the solubilization of some of the protein components of beef heart mitochondria. The basis of this observation, in addition to the previous evidence given for protein-detergent interactions, is the fact that Criddle et al. (6) found that 0.0005 M sodium dodecyl sulfate was very effective in depolymerizing hexameric cytochrome c_1 into a soluble monomer. This concentration is approximately one-sixteenth the

c.m.c. (0.0081 M) reported for dodecyl sulfate by Shinoda *et al.* (29). Also, 0.0035 M sodium dodecyl sulfate (pH 11), which is still well below the c.m.c., was found effective in solubilizing structural protein (6). Therefore, it appears that the free, long-chain electrolyte (R^- or R^+) plays the intimate role in the solubilization of beef heart mitochondrial protein.

A different type of protein solubilization was reported by Putnam and Neurath (27). Their study of the precipitation of proteins by synthetic anionic detergents indicated that the reaction between protein and detergent would occur at pH values on both sides of the isoelectric point. In the reaction of serum albumin with sodium dodecyl sulfate at a pH below the isoelectric point, the formation of a precipitate was observed when the mole ratio of protein to detergent ranged from about 0.01 to 0.02. Klevens (18) attributed the formation of a precipitate to an increase in the hydrophobic nature of this complex due to the presence of the hydrophobic tails of the detergent at the protein interface. Further addition of detergent resulted in a loss of opacity through the formation of another complex (27). Klevens (18) classified this region of "detergent excess", where any previously formed precipitate was dispersed spontaneously or on shaking, as an example of solubilization in that particular system. He stated that this solubilization was probably due to binding of the hydrocarbon tail of the added detergent with the hydrophobic portion of the detergent molecule in the original complex; that is, each hydrophobic tail of the original complex

was replaced with a hydrophilic group. He believed that it is doubtful that association between detergent and protein possibly involves the binding of small ionic micelles. He based this conclusion on the relatively low number of dodecyl sulfate molecules known to be bound per serum albumin molecule, and from the fact that, based on a set of calculations he made, the bound detergent molecule would be too far removed to interact with other detergent molecules in a micelle. Similar evidence was presented by Putnam (26) and Fox and Foster (10), who showed that the amount of detergent bound in a protein-detergent precipitate is stoichiometrically related to the number of oppositely charged groups in the protein. This would appear to rule out the interaction of a micelle, which is believed to be nonuniform in size.

Burkhard and Kropf (5) found that three anionic surfactants (sodium dodecyl sulfate, deoxycholate, and cholate) were very effective as solubilizing agents of beef heart mitochondria, especially dodecyl sulfate and deoxycholate. In their study, no attempt was made to determine the type of protein being solubilized. They found that most of the mitochondrial protein was solubilized after only one treatment with a solution of dodecyl sulfate at a concentration slightly less than its c.m.c. Again it appears that the presence of a micelle is probably not necessary for the solubilization of mitochondrial protein of the beef heart, since no appreciable increase in the solubilization occurred at detergent levels above their respective c.m.c. This

was especially true in the case of sodium cholate and deoxycholate.

Nonionic surfactants have been used to a limited extent to extract and solubilize enzymes from animal tissue. Ord and Thompson (25) used the nonionic agent Lubrol W (cetyl alcohol-polyethylene condensate) to obtain an enzymatically active dispersion of rat brain cholinesterase.

Probably the most commonly used nonionic surfactant is Triton X-100 (polyethylene-t-octylphenylether, $\overline{M}$ = 624).

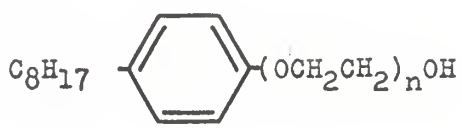

Fig. 1 Triton X-100 (n = 9-10).

Brunngraber and Aguilar (3) found that 15 enzymes were released from a homogenerate of rat brain mitochondria upon treatment with a 0.5% solution of Triton X-100. Walker and Levvy (32) used a 0.12% Triton X-100 solution to release beta-glucuronidase from a homogenate of mouse liver. They also used it (a 0.075% solution was found effective) in their isolation of this enzyme from rat liver (33). Wattiaux and deDuve (34) showed that a 0.1% Triton X-100 solution was as effective in releasing five hydroxylases from rat liver mitochondria as was homogenizing in a Waring blendor for three min. or repeated freezing and thawing

(12 times). A 1% solution of Triton X-100 was found by Feinstein (9) to help solubilize mouse liver catalase. Bendell and deDuve (2) were able to determine that a 0.1% Triton X-100 solution was effective in releasing various dehydrogenases from rat liver mitochondria.

Roodyn (28), in preparing his mitochondrial subfractions, extensively used Triton X-100 [and other nonionics in this series of compounds manufactured by Rohm and Haas Co., Philadelphia, Pa. (31)] as a solubilizing agent. He found that when Triton X-100 was added in sufficient concentration to a suspension of rat liver mitochondria (0.6 mg. protein/ml.), an immediate visual clearing occurred. This "clearing" was coincident with a marked fall in turbidity and with the release of protein and nucleic acid from the mitochondria. He found with a detergent concentration of 0.04% (v/v) that only approximately 20% of the mitochondrial protein (micro-Kjeldahl nitrogen equivalent) was found in a pellet after centrifugation at 10,000 x g. He noted that, if a sufficient concentration of detergent was used, the fall in turbidity took place in less than five sec., indicating an almost instantaneous lysis of the mitochondria. With lower concentrations of detergent, however, this immediate rapid fall was followed by a slower fall lasting 40-50 min. Also, for a given concentration of detergent, Roodyn noted that the extent of disruption was affected by the concentration of the mitochondrial suspension, the less concentrated suspensions being more easily disrupted. In addition, the degree of disruption was also

affected by the length of the polyoxyethylene chain in the Triton detergent, Triton X-100 (with 9-10 ethylene oxide groups, see Fig. 1) and Triton X-102 (with 12-13 groups) being more effective, volume for volume, than Triton X-165 (16 groups) and Triton X-305 (30 groups). The long-chain detergents also were less effective on a molar basis; that is, 0.2% Triton X-165 (2.22 mM) was considerably less effective than 0.1% Triton X-100 (1.54 mM). To summarize, Roodyn found that the extent of disruption of rat liver mitochondria by Triton X-100 could be controlled by the detergent concentration, the mitochondrial concentration, the length of time the mitochondria were exposed to detergent, and the length of the polyoxyethylene chain.

A preliminary study made in this lab (7) indicated that beef heart mitochondria were not as effectively solubilized by Triton X-100 as were the rat liver mitochondria in Roodyn's study (28). Therefore, it was decided to undertake a study of the solubilization of beef heart mitochondria by four Tritons (X-45, n=5; X-100, n=9-10; X-205, n=20; and X-305, n=30), following the same procedure of Burkhard and Kropf (4). In addition, we decided to follow rat liver mitochondrial solubilization by Triton X-100 (using the same procedure as in the beef heart study) to see if we could correlate our work with that of Roodyn (28).

EXPERIMENTAL

Isolation of Mitochondria

Beef heart mitochondria were isolated according to the procedure of Green and Zeigler (13). The hearts were trimmed of excess fat and connective tissue, cut up and passed through a cold meat grinder. The ground muscle was washed four times with five volumes of 0.25 M sucrose containing 0.001 M ethylenediaminetetraacetate (EDTA). Two hundred grams of the washed mince were suspended in 500 ml. of 0.25 M sucrose and homogenized in a Waring blendor at top speed for 20 sec. The homogenate was centrifuged at 1,000 x g for 10 min. to sediment the cell debris, nuclei, etc. The supernate was carefully decanted and centrifuged at 15,000 x g for 10 min. The mitochondrial pellet was thoroughly homogenized with a Potter-Elvehjem homogenizer in 10 volumes of 0.25 M sucrose containing 0.001 M Tris buffer (pH 7.8). After centrifuging the suspension at 12,000 x g for 10 min., the residue was washed in 10 volumes of 0.25 M sucrose. The pellet was resuspended in four volumes of 0.25 M sucrose, analyzed for protein content, and an aliquot containing approximately 40 mg. of protein was measured into a set of test tubes. The required aliquot contained, on the average, three to four ml., and was subsequently frozen until used.

Rat liver mitochondria were prepared according to a modified procedure of Weinbach (35). The livers of eight adult albino rats were quickly excised and chilled in cold 0.25 M

sucrose. The tissue was cut up and homogenized with nine volumes of cold 0.25 M sucrose in a micro-Waring blendor. The suspension was then thoroughly homogenized in a Potter-Elvehjem homogenizer, and centrifuged at 600 x g for 10 min. The supernate was decanted and centrifuged at 8,500 x g for 10 min. The pellets were washed in 0.25 M sucrose, and resuspended in sufficient 0.25 M sucrose to give 20 mg. of protein per ml. As with the beef heart mitochondria, approximately 40 mg. of mitochondrial protein were measured out and frozen until used.

Biuret Determination of Protein

Protein in both the beef heart and the rat liver mitochondrial preparations was determined by the biuret method using the reagent of Gornall, Bardawill, and David (11). Their procedure was modified in the following manner; six ml. of the reagent were added to the protein sample and the total volume was adjusted to 12 ml. with distilled water. This solution was placed in a boiling water bath for one min. and then cooled to room temperature in an ice bath. The resulting absorbance was determined in a 1.2 cm. cell at 540 mmu in a Bausch and Lomb Spectrophotometer exactly 10 min. after heating. A standard curve was prepared using crystalline bovine serum albumin (Fig. 2).

Folin-Ciocalteu Determination of Protein

Protein in solutions from the solubilization studies was determined, as much as possible, using the Folin-Ciocalteu

reagent (Calbiochem) in the manner outlined by Litwack (20). The following modifications were made: 10 ml. of the alkaline copper solution were added to the protein sample and the total volume was adjusted to 11 ml. with distilled water. This solution was warmed in a water bath at 38°C. for 15 min. One ml. of diluted Folin-Ciocalteu reagent (FCR) was added, and after 15 min. of color development, the absorbances of the resulting solutions were then determined in a 1.2 cm. cell at 660 mmu in a Bausch and Lomb Spectronic 20 Spectrophotometer. For the first few determinations using the Folin-Ciocalteu method, one part of the stock FCR was diluted with only one part of water. This dilution was later found to be in error, at least in accordance with the modified Folin-Ciocalteu reagent used by Lowry et al. (21). The correct dilution was one part stock FCR plus two parts of water, and this dilution was used for the remainder of the Folin-Ciocalteu determinations. Therefore, two calibration curves were required; tyrosine was used as the standard in both cases (Fig. 3).

Nitrogen Determination

Protein solutions containing high concentrations of either salt or detergent caused difficulties in the Folin-Ciocalteu determinations, so the extent of solubilization was followed by performing nitrogen determinations using the micro-Kjeldahl method as adapted from the procedures of Niederl and Niederl (24) and Thompson and Morrison (30). Sulfuric acid (50%) and

a potassium sulfate-copper sulfate catalyst were used for the digestion which was carried out for 15-20 min. Any remaining color was removed by oxidation with a small amount of 30% hydrogen peroxide. The ammonia released in the distillation step was determined colorimetrically by use of Nessler's reagent prepared as outlined in Harrow et al. (14), and following the safeguards described by Thompson and Morrison (30). The absorbances of the solutions were determined in one cm. quartz cells on a Beckman DU spectrophotometer at 430 mmu after allowing 20 min. for color development. A standard curve was prepared using ammonium sulfate (Fig. 4).

Solubilization Procedure

The procedure followed was that of Burkhard and Kropf (4). Mitochondria which had been previously isolated and frozen in small aliquots (approximately 40 mg. of protein) were washed once in cold 0.25 M sucrose to remove any soluble protein which might be present due to cell disruption caused by the freezing procedure. (At first, two washes were performed but this was thought to be unnecessary since very little, if any, protein was found in the second wash.) Fifteen ml. of cold (0°C.) solvent (e.g. surfactant solution) was added to the freshly sedimented pellet, and this system was homogenized with a Potter-Elvehjem homogenizer. Approximately three ml. of this suspension was saved for later analysis, with the remainder being centrifuged for 10 min. at 100,000 x g in a Spinco Model L centrifuge set

at 5°C. The supernate was poured off and saved for later analysis. This operation was repeated twice; that is, fresh solvent was added to the residue of each successive centrifugation, making a total of three treatments on the same mitochondria. Protein which was not sedimented under these conditions was defined as solubilized protein.

Protein in solutions from the solubilization studies was determined, whenever possible, by use of the Folin-Ciocalteu reagent. The micro-Kjeldahl method of determining total nitrogen was used to estimate the amount of protein nitrogen in solutions where the Folin-Ciocalteu method was not feasible. Solubilized protein (or protein nitrogen), relative to that originally in the mitochondrial suspension, was expressed as a percentage:

$$\frac{\text{total solubilized protein}}{\text{total protein}} \times 100 = \text{percentage solubilized.}$$

RESULTS AND DISCUSSION

Comparison of the Various Methods of Analysis

The correlation coefficients and absorbancy indices of the two tyrosine standard curves were calculated by the Least Squares method. A correlation coefficient of greater than 0.9 was obtained for both curves. The calculated molar absorbancy indices were slightly different, 1.19 (10^4) liter/mole-cm. for curve A and 1.05 (10^4) liter/mole-cm. for curve B in Fig. 3. For curve A an absorbance of 0.25 corresponded to 35 mg. of tyrosine, while with curve B this absorbance would correspond to 40 mg. Since both the supernate and homogenate for a given system were analyzed with the same diluted FCR, no error was thought to have been introduced due to the differences in diluting the stock FCR.

One mg. of beef heart mitochondrial nitrogen (micro-Kjeldahl) was found to be equivalent to 6.8 mg. of bovine serum albumin (biuret) or 1.7 mg. of tyrosine (Folin-Ciocalteu). In the case of rat liver mitochondria, one mg. of mitochondrial nitrogen (micro-Kjeldahl) was equivalent to 1.6 mg. of tyrosine (Folin-Ciocalteu) or 7.6 mg. of bovine serum albumin (biuret). The differences observed between rat liver and beef heart mitochondria were consistent with the findings of Klaassen (17), who found that the insoluble proteins from beef heart mitochondria contain slightly more tyrosine than those from rat liver mitochondria.

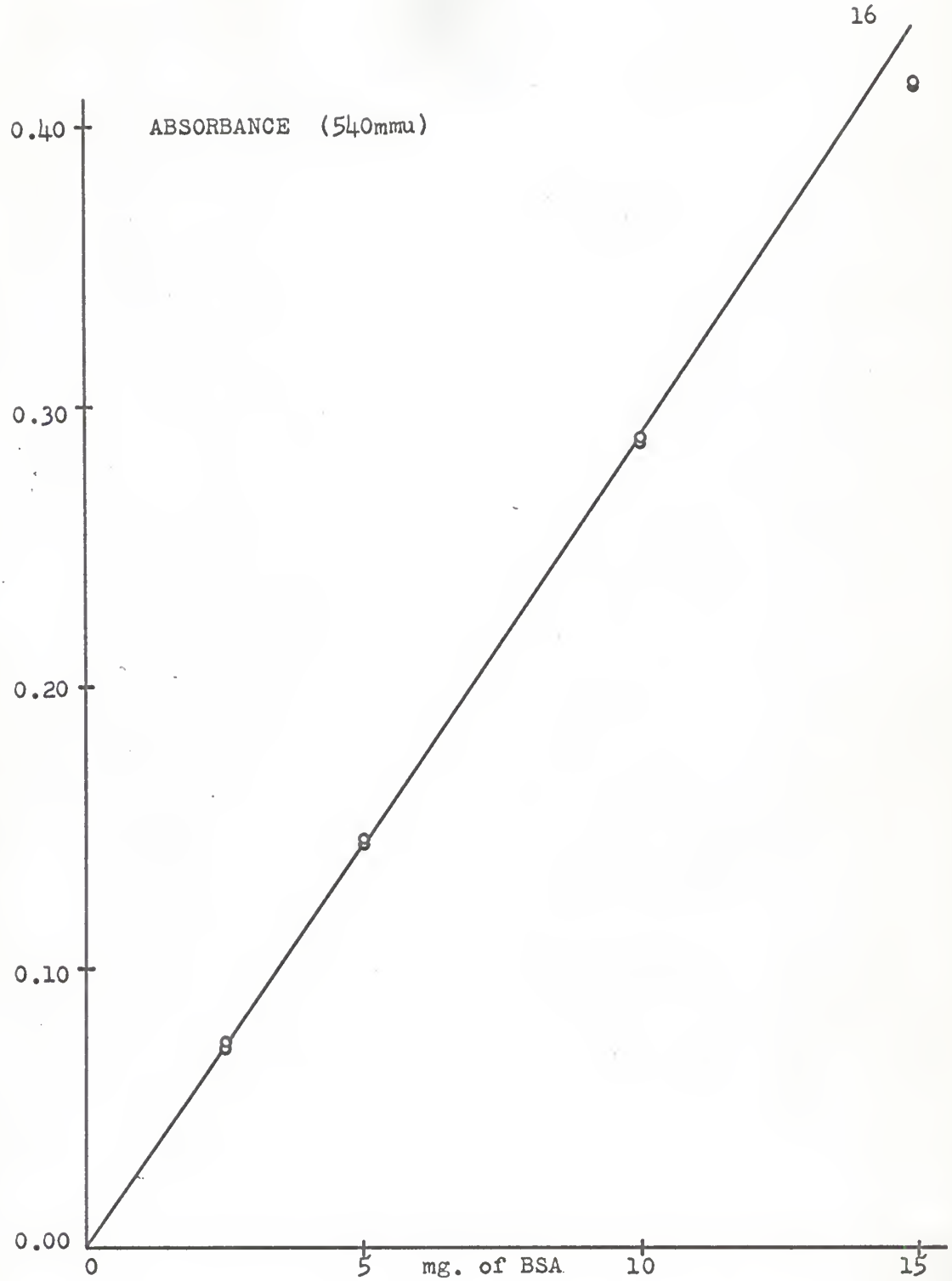

Fig. 2. Standard curve of increasing bovine serum albumin concentration as determined by the biuret reaction.

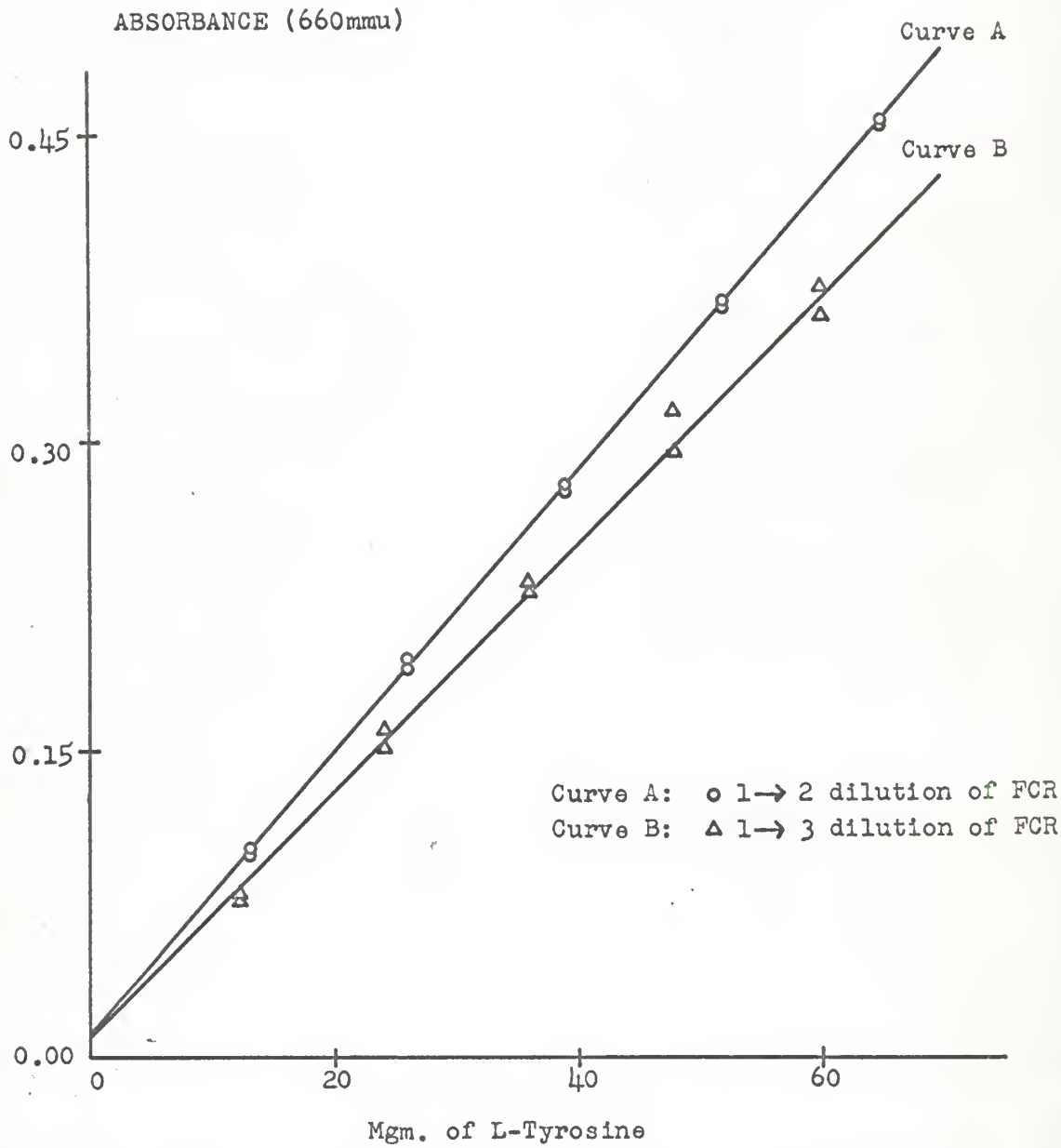

Fig. 3. Standard curves of increasing tyrosine concentration as measured by the Folin-Ciocalteu reaction.

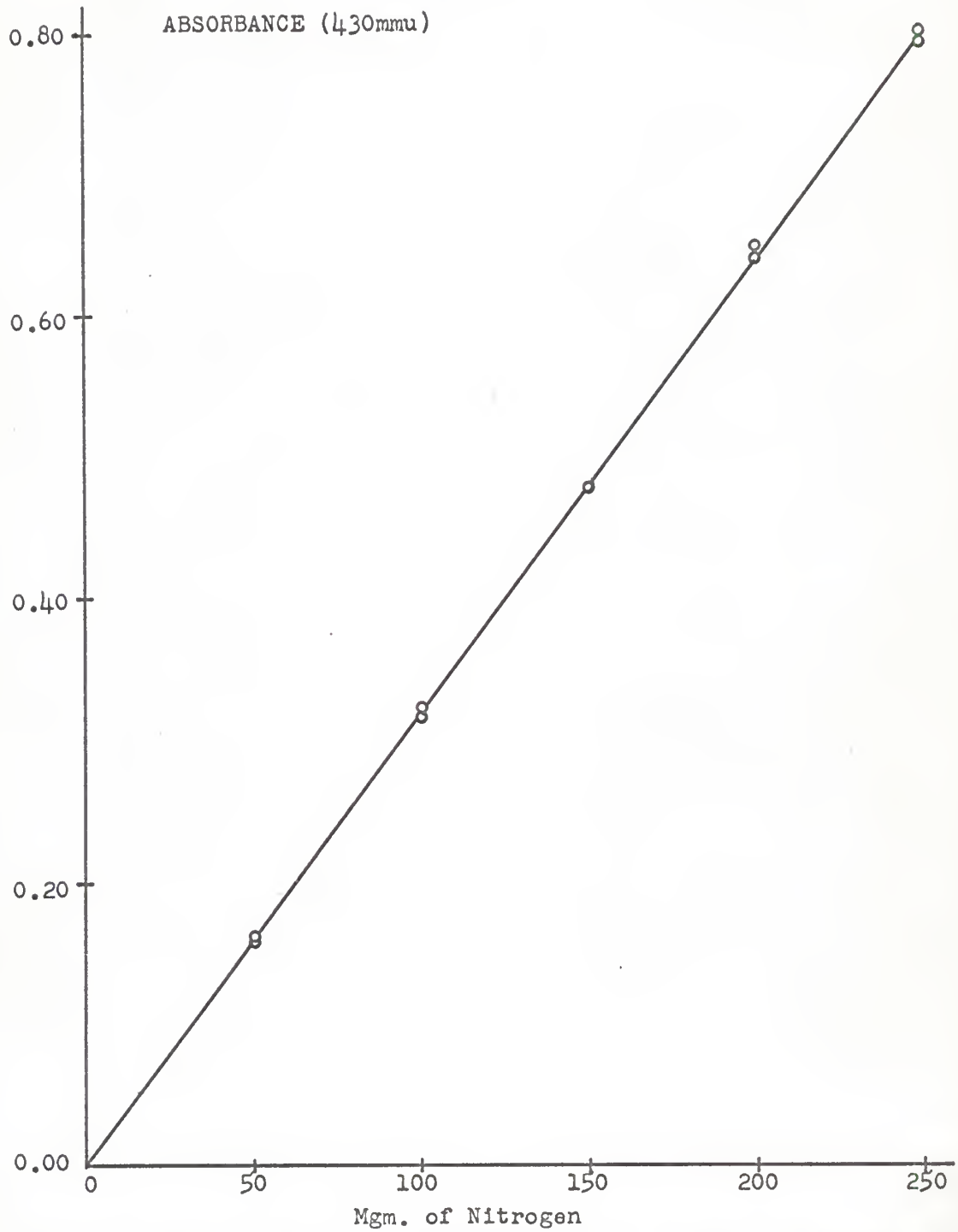

Fig. 4. Standard curve for determining the nitrogen in ammonium sulfate as measured by use of Nessler's reagent.

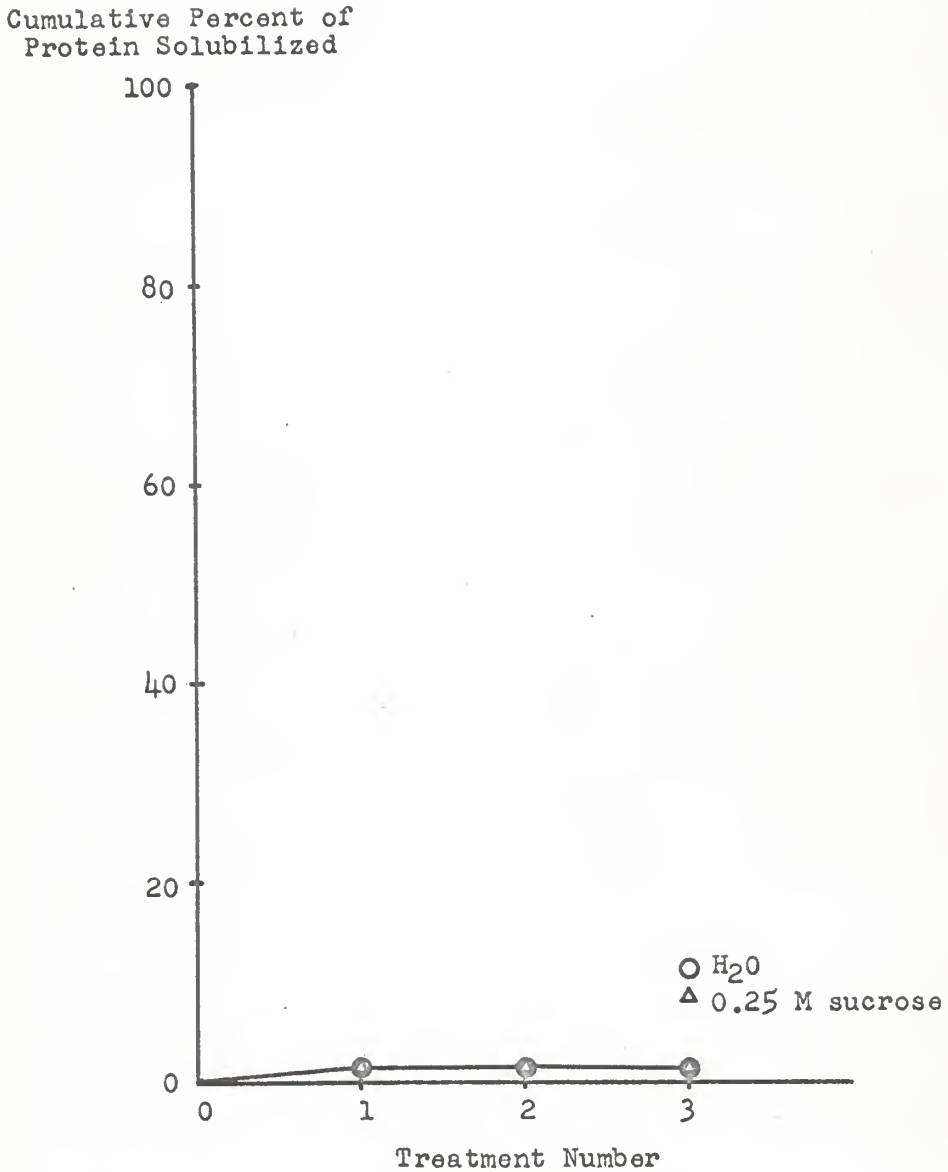

Fig. 5. Solubilization of beef heart mitochondrial protein by three successive treatments with either water or 0.25 M sucrose.

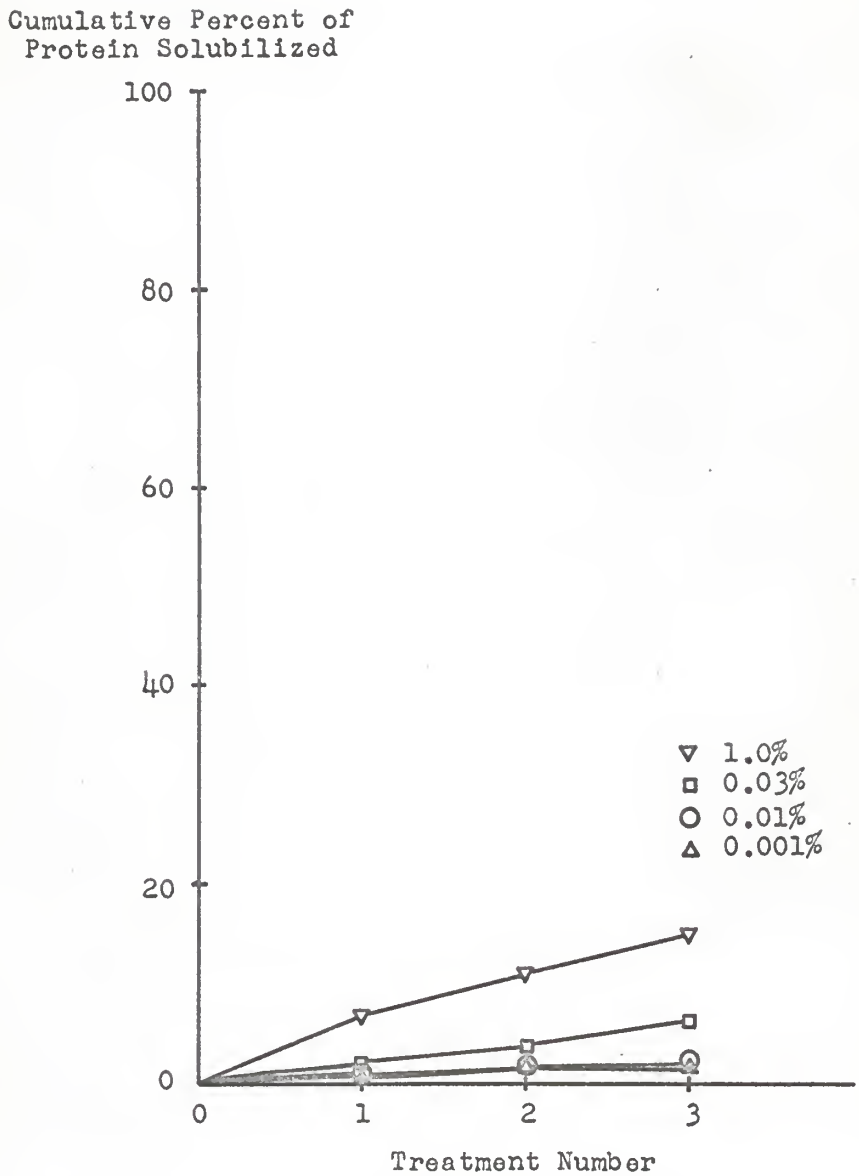

Fig. 6. Solubilization of beef heart mitochondrial protein by three successive treatments with solutions of Triton X-45.

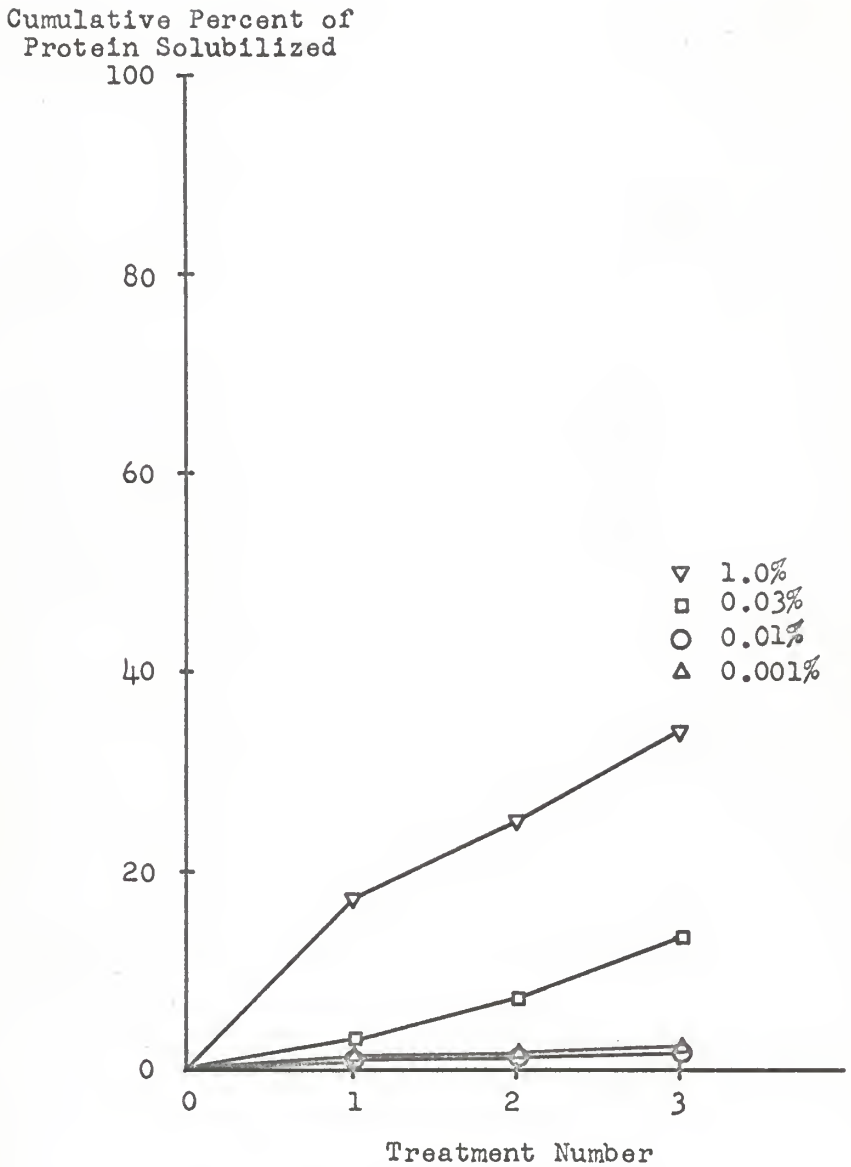

Fig. 7. Solubilization of beef heart mitochondrial protein by three successive treatments with solutions of Triton X-100.

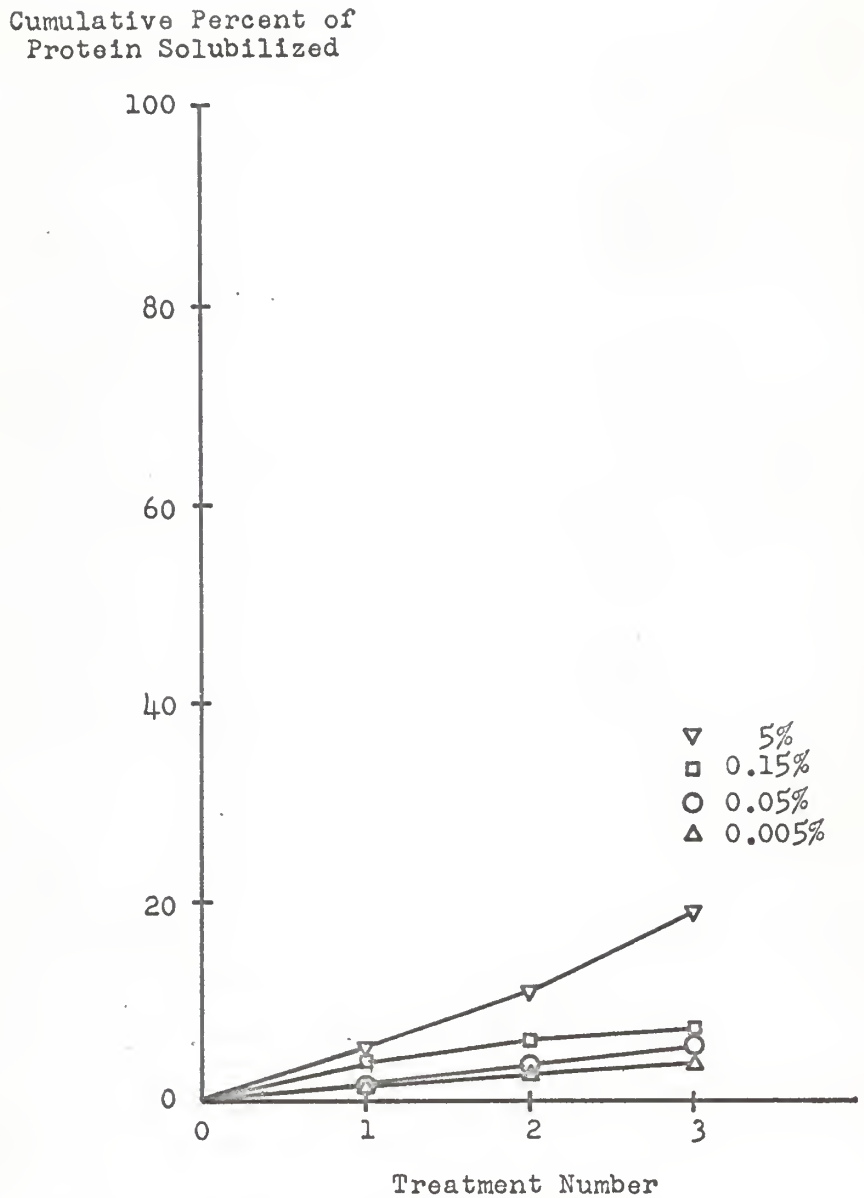

Fig. 8. Solubilization of beef heart mitochondrial protein by three successive treatments with solutions of Triton X-205.

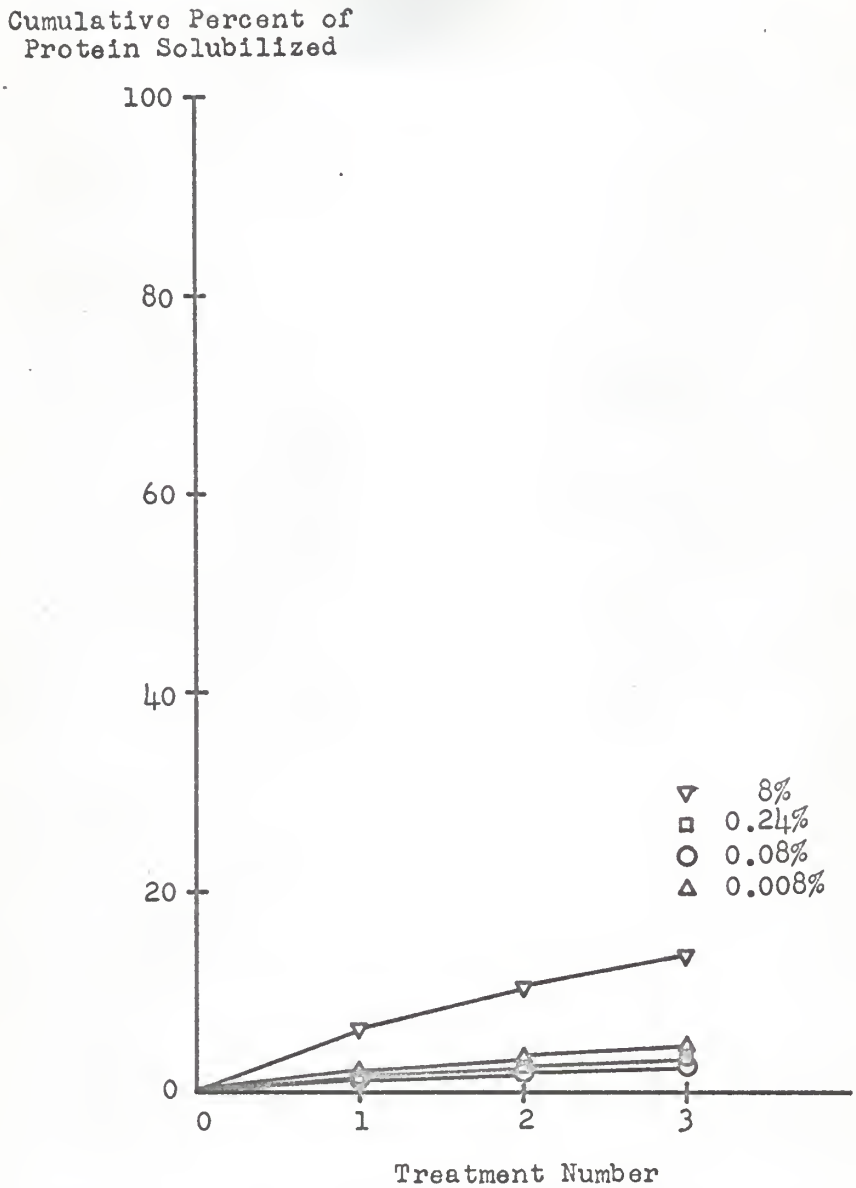

Fig. 9. Solubilization of beef heart mitochondrial protein by three successive treatments with solutions of Triton X-305.

Solubilization of Beef Heart Mitochondria

Water or 0.25 M sucrose was found to solubilize very little beef heart mitochondrial protein (Fig. 5). Therefore, it was concluded that any additional solubilization observed in the presence of various Triton solutions was due to the surfactant rather than as the result of the manipulative procedure. None of the four Triton surfactants were found to be very effective in solubilizing beef heart mitochondrial protein, at least at the concentrations used in our study (Figs. 5-9). The surfactant concentrations ranged from 0.1, 1.0, 3.0, and 100 times the reported critical micelle concentrations for the respective Tritons (31). These values were (weight percent): Triton X-45, 0.005-0.01% ($1.16\text{-}2.32 \times 10^{-4}$ M); Triton X-100, 0.01% (1.58×10^{-4} M); Triton X-205, 0.05% (3.22×10^{-4} M); and Triton X-305, approximately 0.08% (3.66×10^{-4} M). As can be seen from studying Figs. 6-9, very little protein was solubilized below the respective c.m.c.'s of the four Tritons. Also, levels above the c.m.c. did not appreciably increase the amount of solubilization. Of the four, Triton X-100 was found to be the most effective as a solubilizer, especially at the upper two levels (Figs. 6-9).

Burkhard and Kropf (5) found that by adding KCl to solutions of sodium cholate and deoxycholate, they could effectively increase the solubilization by these two anionic detergents. This same effect was observed with the nonionic detergent, Triton X-100. Saturated and 1% KCl were both found to solubilize more protein than water (Fig. 10). With samples where a

saturated KCl-0.03% Triton X-100 solution was used, all the protein was solubilized after two treatments, which was nearly a 50-fold increase in the amount of solubilization when compared to that in the absence of salt (Fig. 11). Even a 1% level of KCl was found markedly to increase the amount of solubilization by either 0.001 or 0.03% Triton X-100 (Fig. 12). In all cases, the KCl-Triton X-100 system solubilized more protein than the sum of the separate Triton and KCl systems alone.

Solubilization of Rat Liver Mitochondria

Water, 0.25 M sucrose, various concentrations of Triton X-100 (1, 3, and 10 x c.m.c.), 1% KCl, and 1% KCl-0.03% Triton X-100 were all found to solubilize a fair amount of rat liver mitochondrial protein (Figs. 13-15), at least when compared to the beef heart study. Also, the 1% KCl-0.03% Triton X-100 system was considerably more effective as a solubilizer than either the KCl or Triton by itself (Fig. 15).

As solubilizers of rat liver mitochondrial protein, none of the Triton X-100 levels used in our studies were found as effective as those reported by Roodyn (28). Part of the differences are probably due to variations in experimental procedures; for example, it appears that Roodyn worked with much more dilute suspensions of rat liver mitochondria. With this in mind, 0.1 and 0.5 the normal starting amount of mitochondrial protein was treated with 0.03% Triton X-100 (two trials each). From these four trials (see Appendix) it appears that decreasing the

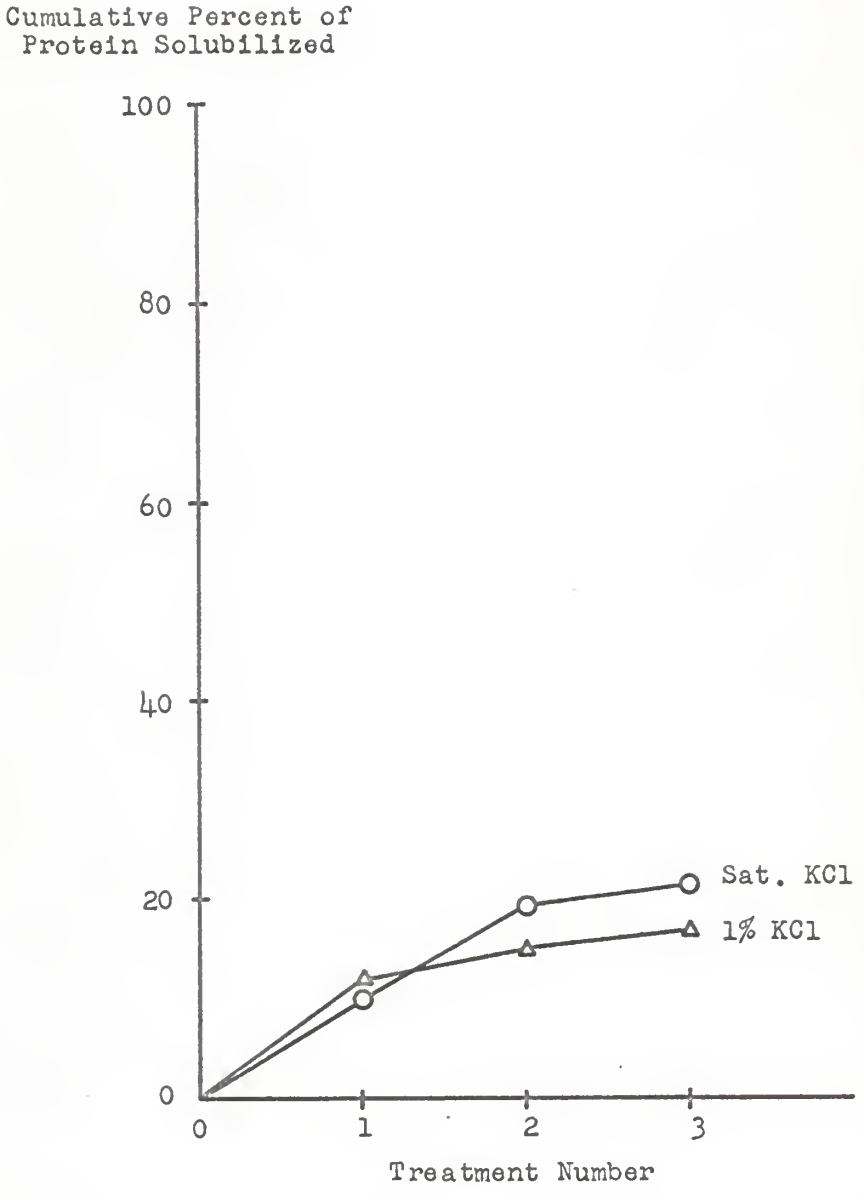

Fig. 10. Solubilization of beef heart mitochondrial protein by three successive treatments with either 1% or saturated KCl (approx. 22%).

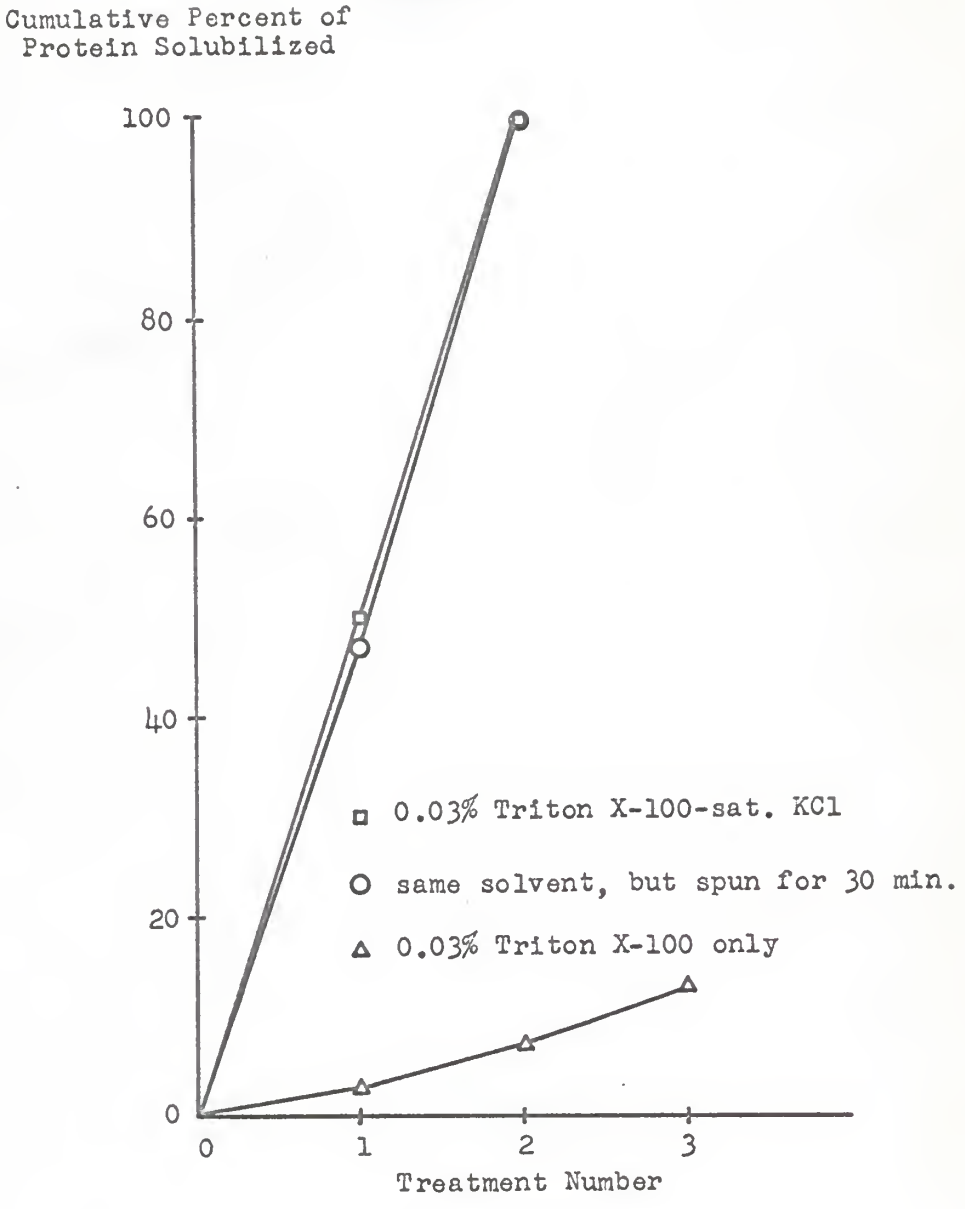

Fig. 11. Solubilization of beef heart mitochondrial protein by three successive treatments with 0.03% Triton X-100-sat. KCl.

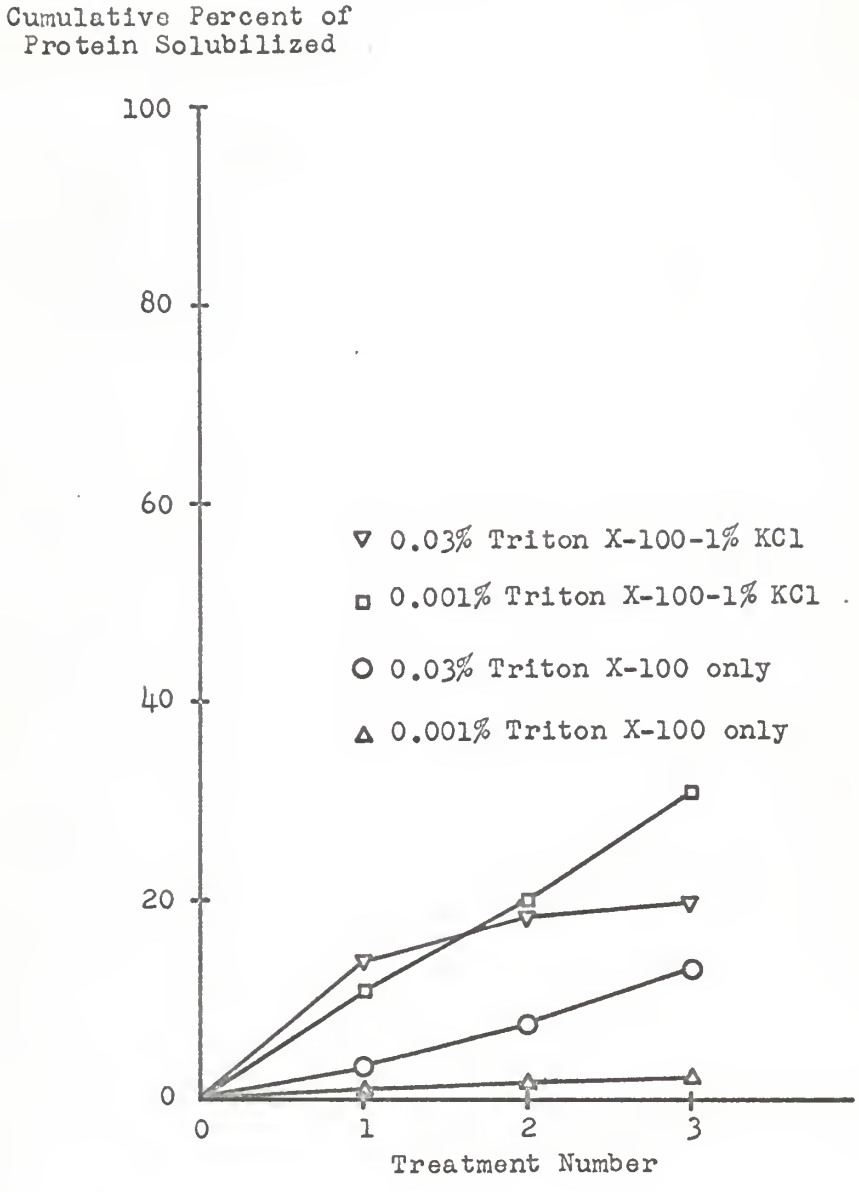

Fig. 12. Solubilization of beef heart mitochondrial protein by three successive treatments with either 0.001 or 0.03% Triton X-100-1% KCl.

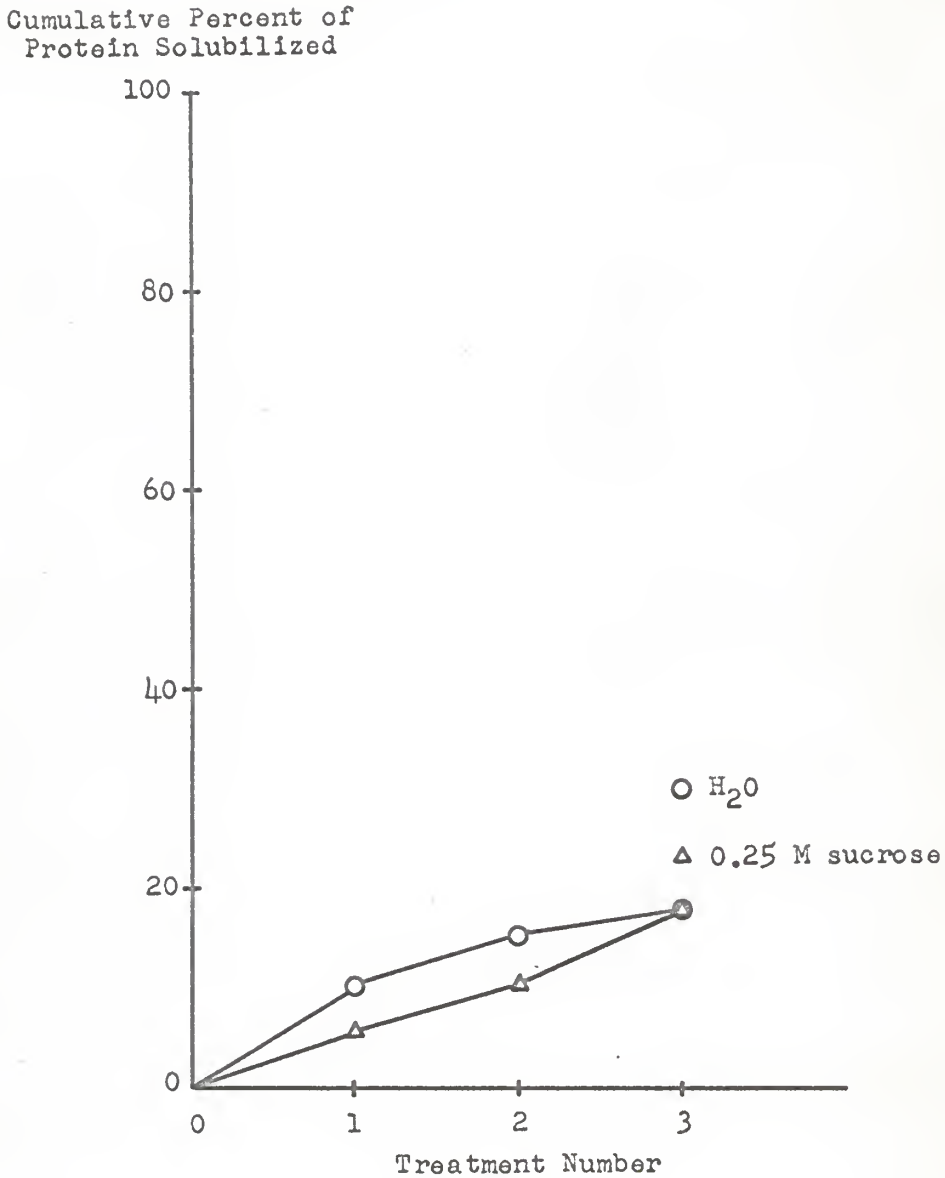

Fig. 13. Solubilization of rat liver mitochondrial protein by three successive treatments with either water or 0.25 M sucrose.

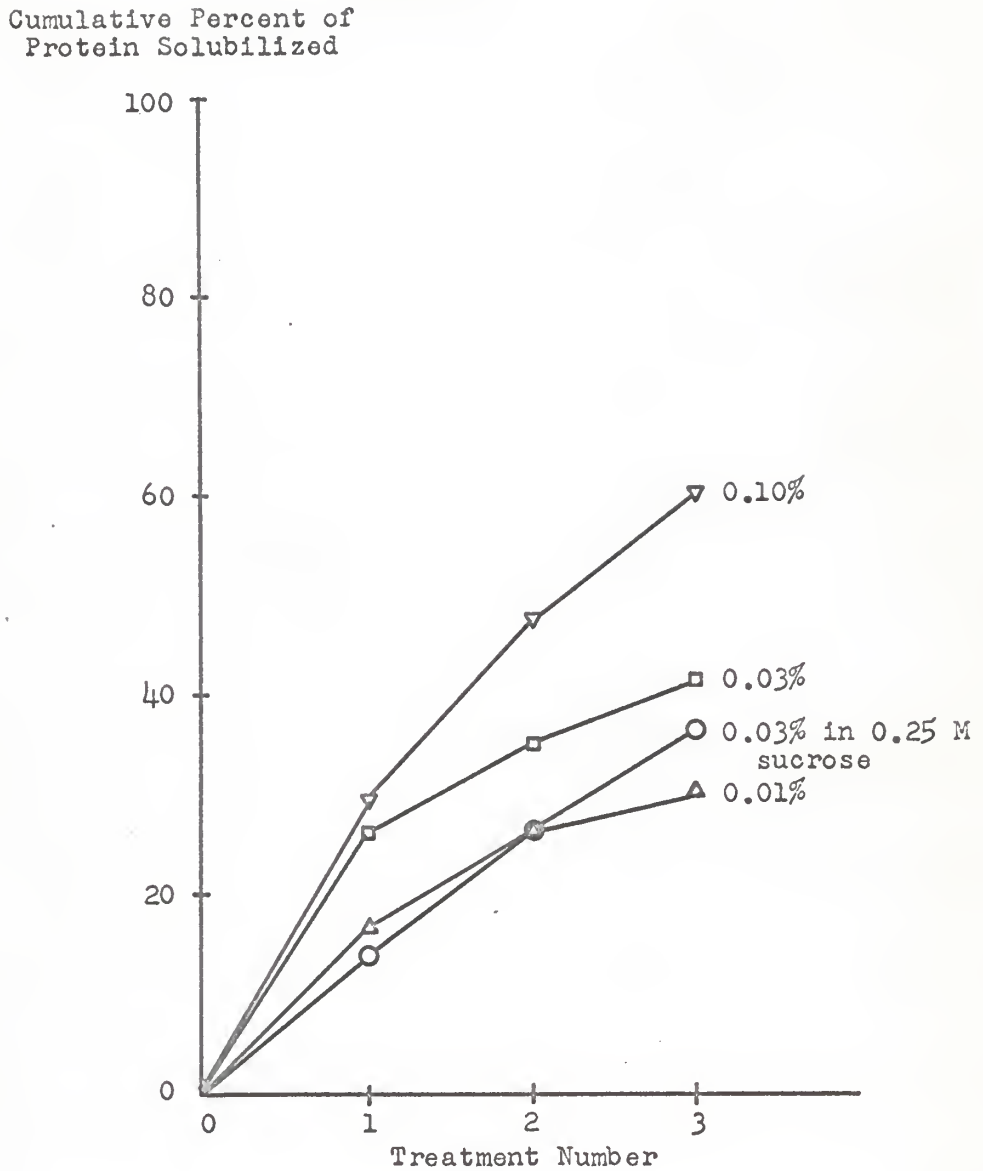

Fig. 14. Solubilization of rat liver mitochondrial protein by three successive treatments with solutions of Triton X-100.

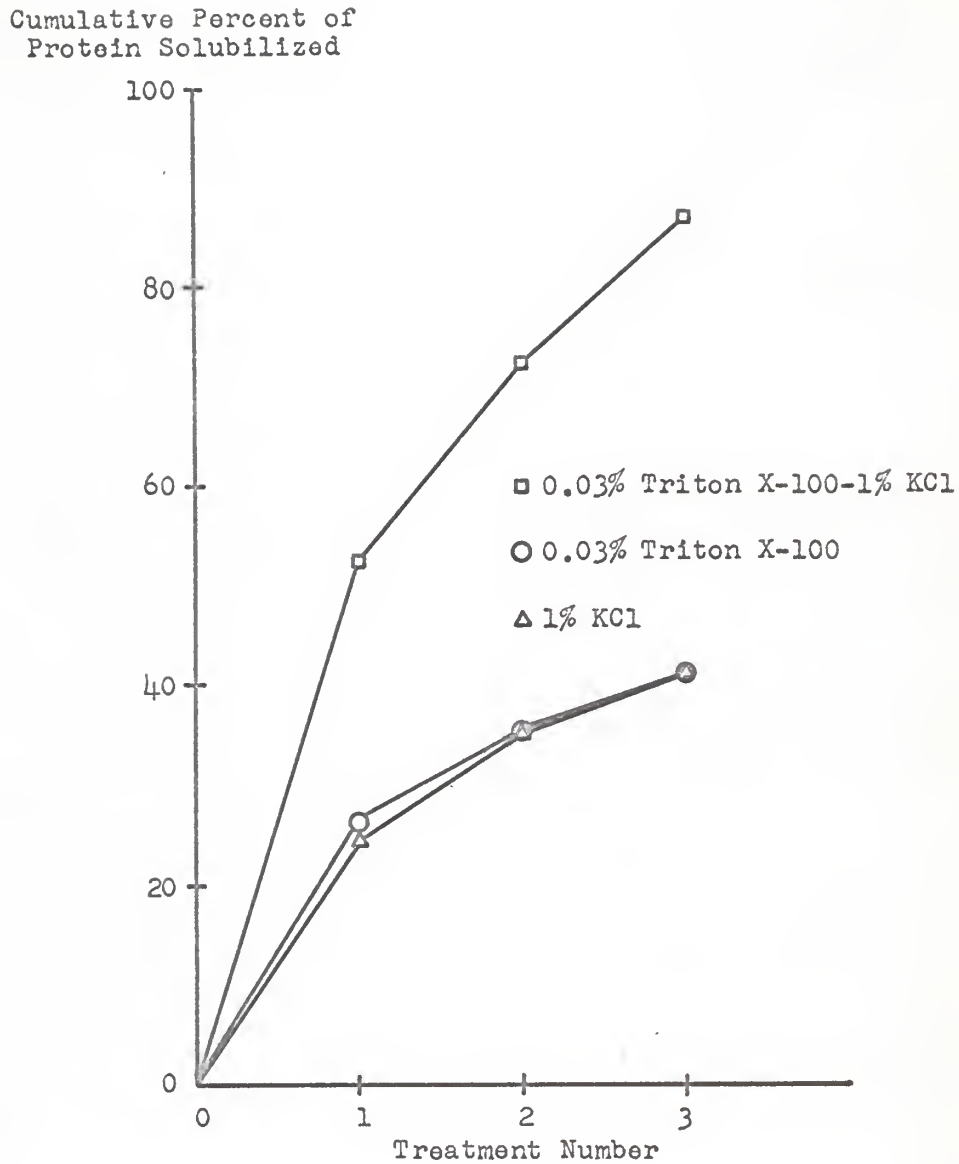

Fig. 15. Solubilization of rat liver mitochondrial protein by three successive treatments with either 1% KCl or 0.03% Triton X-100-1% KCl.

starting amount of protein has no appreciable effect on the percentage solubilized, at least not with our procedure. This also differed from Roodyn's work (28), where he found that dilute suspensions were more easily disrupted.

The presence of an unmentioned electrolyte in Roodyn's suspensions could be a possible explanation of the differences observed, since we found that 1% KCl-0.03% Triton X-100, with just one treatment, solubilized approximately 50% of the mitochondrial protein (Fig. 15). This value was comparable to that obtained by Roodyn (28) with the same concentration of detergent. In addition, our experimental procedures varied in many respects. In Roodyn's study, mitochondria were suspended in 0.3 M sucrose and rapidly mixed with a suitable volume of eight percent (v/v) Triton X-100 in 0.3 M sucrose. The degree of disruption of the mitochondria was followed by measuring the fall in turbidity of the treated suspension. The turbidities were immediately read in an EEL nephelometer, the instrument being set to 100 with a suspension of untreated mitochondria. Also, in some experiments the degree of disruption was measured by immediately centrifuging the suspensions at 10,000 x g for 30 min., and analyzing the sediment for protein or total nitrogen (28). These variations no doubt could be responsible for some of the observed differences.

Comparison of the Solubilization of Beef Heart and Rat Liver Mitochondria

In all cases Triton X-100 and any other solvent system tested were found to solubilize much more rat liver mitochondrial

protein than beef heart. Thus, it would appear that there must be some definite structural difference between the two types of mitochondria. Green (12) stated that heart mitochondria have relatively few cristae. Related to the number of cristae is the ratio of particle-bound protein to soluble protein. Green's data indicate that there is a direct relationship between the number of cristae and the amount of bound protein. The following figures were given by Green (12): particle-bound protein, heart (82%) and liver (40%); and soluble protein, heart (18%) and liver (60%). Assuming that these figures are reliable, an explanation for the greater solubilization of rat liver mitochondrial protein than beef heart can be given. It appears that once the membrane is disrupted by means of a surfactant solution, etc., more than 50% of the rat liver mitochondrial protein would be released and pass into solution, whereas in the case of beef heart mitochondria only about 20% of this protein would be released. Obviously this is an oversimplification of a more complicated interaction. Quite possibly there could be a significant difference in the membrane which surrounds the mitochondrion of these two tissues; according to Lehninger (19) there are some differences in the phospholipid content of these two types of mitochondrial membranes. It seems probable, though, that this approach is a starting point when comparing the solubilization of these two proteins.

Effect of Added Electrolyte on the Solubilization of Mitochondrial Protein by Nonionic Detergents

From the results given in Figs. 11, 12, and 15, the presence of electrolyte obviously plays an important role in the solubilization of mitochondrial protein by nonionic detergents. Criddle and co-workers (6) observed a somewhat similar effect in their study of the proteins of the electron transfer system. They found that both 1% Triton X-100 and 0.05 M thioglycolate ($HS-CH_2-COO^-$) were ineffective in depolymerizing cytochrome c_1. When 0.05 M thioglycolate was used in conjunction with the nonionic detergent (0.01%), they did obtain the monomeric species. To explain these observations they attributed the success of the combination to two things, namely: thioglycolate is capable of introducing a charged group into a few key positions in the cytochrome c_1 hexamer, whereas the hydrocarbon side chain of Triton X-100 is able to compete with the cytochrome c_1 molecules for the individual hydrophobic binding sites. This explanation was based on the one they had given to account for the effectiveness of the anionic detergents in breaking up complexes composed of various electron transport particles. That is, anionic detergents possess both the capability of hydrophobic competition and for the introduction of a large number of like charges, which leads to a resultant charge repulsion and eventual breakdown of the various complexes.

To account for the effect of the added electrolyte, several explanations have been proposed. One includes the possibility

that the added electrolyte enhances the hydrophobic bonding between the protein and the hydrocarbon side chain of Triton X-100. If this interaction between the mitochondrial protein and the surfactant is increased, there should be a concomitant increase in solubilization. Another possible explanation may involve the familiar "salting-in" phenomenon (36) observed when neutral salts are added to a protein solution. Whatever the actual effect is, though, it seems certain that it is a combination of both the added electrolyte and detergent (see Figs. 10, 11, 12, and 15), and not just due to the salt. Therefore, it appears that this effect cannot be adequately explained until further investigations are carried out on the actual solubilization mechanism.

SUMMARY

The solubilization of beef heart mitochondrial protein by four nonionic surfactants (Tritons X-45, X-100, X-205, and X-305) was investigated, both in the presence and absence of salt (KCl). A similar study was made on rat liver mitochondria, using only Triton X-100.

None of the four Triton surfactants, at concentrations used in this study were found to be effective in solubilizing beef heart mitochondrial protein. An attempt was made to account for the effect of the added salt, but it was thought that no definite role could be assigned at this time.

All the solvent systems studied were found to solubilize considerably more rat liver mitochondrial protein than beef heart. Although no definite conclusion can be made to explain these observed differences, it was suggested that known variations in the structures of these two types of mitochondria could possibly account for them.

Triton X-100 was not observed to be as effective a solubilizer for rat liver mitochondria as was reported by Roodyn (28). Variations in the experimental procedures were thought to account for part or all of these differences, though.

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A P P E N D I X

SOLUBILIZATION OF BEEF HEART MITOCHONDRIA

System	Treatment Number	Percent Solubilized
H_2O	1	0.7, 1.3
	2	0.4, 0.0
	3	0.0, 0.0
0.25 M Sucrose	1	0.9, 0.9
	2	0.5, 0.0
	3	0.0, 0.0
0.001% Triton X-45	1	0.7, 0.7
	2	0.9, 0.6
	3	0.0, 0.0
0.01% Triton X-45	1	0.4, 0.7
	2	0.9, 0.8
	3	0.6, 0.5
0.03% Triton X-45	1	1.4, 1.6
	2	2.5, 2.0
	3	2.6, 2.9
1.0% Triton X-45	1	6.3, 5.4
	2	5.5, 5.0
	3	4.2, 3.7
0.001% Triton X-100	1	1.3, 1.0
	2	0.8, 0.6
	3	0.9, 0.0
0.01% Triton X-100	1	0.8, 0.6
	2	0.2, 0.7
	3	0.4, 0.3
0.03% Triton X-100	1	3.3, 2.7
	2	3.5, 5.2
	3	6.3, 5.2
1.0% Triton X-100	1	15.9, 18.5
	2	5.9, 9.6
	3	10.9, 7.4
0.005% Triton X-205	1	1.2, 1.7
	2	1.0, 1.2
	3	1.2, 1.5

SOLUBILIZATION OF BEEF HEART MITOCHONDRIA (cont.)

System	Treatment Number	Percent Solubilized
0.05% Triton X-205	1	1.1, 1.2
	2	2.0, 2.2
	3	2.4, 2.0
0.15% Triton X-205	1	4.4, 4.0
	2	1.9, 1.4
	3	0.8, 0.7
5.0% Triton X-205	1	5.4, 4.0
	2	7.8, 4.3
	3	4.2, 11.6
0.008% Triton X-305	1	2.1, 1.6
	2	1.7, 1.2
	3	1.0, 1.1
0.08% Triton X-305	1	1.1, 1.0
	2	0.7, 0.9
	3	0.6, 0.4
0.24% Triton X-305	1	1.4, 2.1
	2	0.8, 0.5
	3	0.7, 0.0
8.0% Triton X-305	1	5.3, 6.2
	2	4.3, 4.5
	3	3.0, 3.4
1% KCl	1	12.1, 11.9
	2	3.1, 3.0
	3	1.8, 1.6
0.001% Triton X-100 in 1% KCl	1	13.8, 13.8
	2	3.1, 5.4
	3	2.2, 1.3
0.03% Triton X-100 in 1% KCl	1	11.8, 10.0
	2	9.1, 9.1
	3	10.3, 11.4
Saturated KCl	1	7.4, 12.7
	2	10.1, 8.6
	3	1.3, 2.8

SOLUBILIZATION OF BEEF HEART MITOCHONDRIA (cont.)

System	Treatment Number	Percent Solubilized
0.03% Triton X-100 in saturated KCl	1	48.0, 52.0
	2	85.0, 100.0
0.03% Triton X-100 in saturated KCl (spun for 30 min.)	1	39.0, 55.0
	2	100.0, 77.0

SOLUBILIZATION OF RAT LIVER MITOCHONDRIA

System	Treatment Number	Percent Solubilized
H_2O	1	10.0, 10.0
	2	5.9, 5.1
	3	2.8, 2.6
0.25% M Sucrose	1	6.4, 5.2
	2	4.6, 4.3
	3	8.2, 7.0
0.01% Triton X-100	1	17.6, 16.0
	2	9.1, 7.8
	3	5.6, 5.0
0.03% Triton X-100	1	27.6, 24.7
	2	8.1, 10.0
	3	6.8, 5.8
0.03% Triton X-100 in 0.25 M Sucrose	1	14.6, 13.2
	2	13.3, 12.1
	3	10.2, 9.5
0.10% Triton X-100	1	28.6, 29.9
	2	18.8, 17.6
	3	11.7, 13.9
1% KCl	1	25.8, 23.1
	2	9.4, 12.4
	3	6.4, 5.9
0.03% Triton X-100 in 1% KCl	1	54.1, 51.1
	2	18.9, 20.0
	3	14.6, 15.2
0.03% Triton X-100 (0.1 starting protein)	1	21.8, 21.1
0.03% Triton X-100 (0.5 starting protein)	1	18.9, 15.7

THE SOLUBILIZATION OF MITOCHONDRIAL PROTEIN BY NONIONIC SURFACE ACTIVE AGENTS

by

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The solubilization of beef heart mitochondrial protein by four nonionic surfactants (Triton X-45, X-100, X-205, and X-305) was investigated, both in the presence and absence of salt (KCl). A similar study was made on rat liver mitochondria, using only Triton X-100, in an attempt to correlate our results with those reported in the literature.

Mitochondria which had previously been isolated and frozen in small aliquots (approximately 40 mg. of protein) were washed in 0.25 M sucrose, and the freshly sedimented pellet was treated with 15 ml. of solvent. This system was homogenized and the resultant suspension was centrifuged at 100,000 x g for 10 min. This operation was repeated twice on the same mitochondria. Protein which was not sedimented under these conditions was defined as solubilized protein, and this protein, relative to that originally in the mitochondrial homogenate, was expressed as a percentage:

$$\frac{\text{total solubilized protein}}{\text{total protein}} \times 100 = \text{percentage solubilized.}$$

Of the four Triton surfactants tested, X-100 was found to be the most effective in solubilizing beef heart mitochondrial protein, although none of them solubilized very much protein. However, the addition of electrolyte (KCl) to solutions of Triton X-100 markedly increased the amount of solubilization, with all the protein being solubilized after two treatments with saturated KCl-0.03% Triton X-100.

All the solvent systems studied were found to solubilize

a fair amount of rat liver mitochondrial protein, at least when compared to the beef heart study. However, solutions of Triton X-100 were not observed to be as effective in disrupting (and in a sense, solubilizing) rat liver mitochondria as has been reported in the literature. Variations in the experimental procedures were thought to account for part or all of these observed differences.

The presence of electrolyte was found to play an important part in the solubilization of mitochondrial protein by nonionic detergents. Although an attempt was made to account for the effect of the added electrolyte, it was believed that until further investigations are carried out, no definite role can be assigned at this time.